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Cristina Balagna[1,*], Sara Ferraris[1], Sergio Perero[1], Marta Miola[1], Francesco Baino[1], Alfio Battiato[2], Chiara Manfredotti[2], Ettore Vittone[2], Enrica Vernè[1] and Monica Ferraris[1]

1 Institute of Materials Physics and Engineering, Department of Applied Science and Technology,

Politecnico di Torino, 10129, Torino, Italy

2 Experimental Physics Department /Centre of Excellence "Nanostructured Interfaces and surfaces", University of Torino, and INFN sez.Torino Via P. Giuria 1, 10125 Torino, Italy

*corresponding author: cristina.balagna@polito.it

ABSTRACT

Several fields associated to people and everyday life products can be easily affected by bacterial contamination. An increasing interest for antibacterial products can be seen on the scientific literature and on the market in many fields, from medical applications to everyday life products. To this purpose, *a new antibacterial silver nanocluster/silica composite coating* was developed. The radio frequency (RF) co-sputtering is the technique used for the coating deposition, suitable for glasses, ceramics, metals and polymers. Sputtering technology is adaptable and suitable also for substrates susceptible to high temperature (e.g. polymers). Moreover this technology allows the tailoring of the silver content (and consequently the antibacterial activity) depending on the specific application and requirements, from aesthetic appearance to bactericidal power and biocompatibility. The main properties of the coating in terms of antibacterial effect, morphology, composition and adhesion to silica substrates, used as model, will be discussed. In addition, some case studies will be reported, demonstrating the suitability of this coating to be applied on different materials, from medical devices, cheese moulds, mobile telephones to aerospace structures.

1. INTRODUCTION[n1]

1.1 Bacteria can be everywhere: from medical implants to space stations

Bacterial contamination can interest almost every surface from medical implants [1-5] and hospital surfaces (desks, telephones, instruments…) [6-8] to food handling and processing materials [9-11], to everyday life objects [12, 13]; this contamination is not limited to the Earth, in fact microorganisms represent a problem also for space structures [14-18].

In the case of *medical implants*, the development of an infection is a stressful and health hazardous condition that may require post-surgical additional treatments and lead to implant failure and, even removal in the worst cases. Moreover, prosthetic and hospital acquired infections represent a serious problem also from an economical point of view, because of the expensive strategies necessary for their treatment (i.e. additional therapies, revision surgery, longer hospitalization time). Biomaterial surfaces represent a preferential site for bacterial adhesion and biofilm formation [19]. In fact contamination has frequently been registered on orthopedic and dental prostheses [1-3], catheters and abdominal wall repair devices [4, 5] as well as ocular implants [20].

A significant microbial contamination has been observed on frequent hand-touched surfaces in *hospitals* and particularly on telephones of healthcare workers, patients and visitors [6-8]. The contamination from hands to telephones and from telephones to hands has also been noticed [7].
The need of antibacterial surfaces in the medical field comes from both medical devices and clinical environment, with extremely different requirements in terms of compatibility, bactericidal activity and resistance.

If it is straightforward to associate the medical field with the risk of bacterial contamination, it should be also evident that these microorganisms can reside almost everywhere: in particular, every surface at *home* is a possible point of contamination for bacteria (e.g. S aureus and other staphylococci); examples are: working surfaces, sinks, refrigerator and sponges in the kitchen, cabinet top and shelves, hand towels and taps in but also pillows and bed lining, toys, television sets, remote controls, telephones, door knobs and carpets, where people, animals and generally the environment can get contaminated [13].

Food handling and production is another critical source of bacterial contamination that can result in human poison and health concerns [9-11]. Cross contamination of foods from processing materials have been reported for cheese and meat industries [9-11, 21].

A quite unexpected habitat for bacteria is the *space*, but microbial contamination of space structures has been widely documented [15-18, 22] and constitutes a serious problem for astronauts because of their hampered immune system [14, 15] and for the deterioration of the space structures [22, 23].

A wide variety of bacteria have been detected on surfaces for the different application cited above, some examples are reported in Table 1. The variety of bacterial contamination requires a wide spectrum of antibacterial solutions. Moreover the development of resistant bacterial strains require new and low resistant development strategies.

Table [n2]1: Main bacterial and fungal strains for different application fields

Application field	Main pathogens	Reference
Medical	*S. aureus (mainly on metals)*	[2], [4]
	S. epidermidis (mainly on polymers)	[2], [4]
	Streptococci (e.g. S. sanguis, dental)	[3]
	Actinomyces (dental)	[3]
	Porphyromonas (dental)	[3]
	Prevotella (dental)	[3]
	Capnocytophaga (dental)	[3]
	Fusobacterium (dental)	[3]
	P. gingivalis (dental)	[3]
	C. gingivalis (dental)	[3, 24]
	C. albicans (mainly on polymers, dental)	[24-26]
Food handling	*Enterobacter*	[11, 27]
	Lactobacillus	[21, 27]
	Listeria	[9, 21, 27, 28]
	Micrococcus	[27]
	Streptococcus	[27]
	Bacillus	[27]
	Pseudomonas spp	[21, 27]
	E. coli	[9, 11, 21, 28]
	C. jejuni	[28]
	Salmonella	[9]
	S. aureus	[9-11]
Everyday life	*S. aureus and epidermidis (telephones, household)*	[6, 7, 12, 13]
	Acinetobacter spp (telephones)	[7, 8, 12]
	Pseudomonas spp (telephones)	[7, 8, 12]
	Enterococcus spp (telephones)	[8, 12]
	Streptococcus spp (telephones)	[8, 12]
	Escherichia coli (telephones)	[8, 12]
	Klebisella spp (telephones)	[8, 12]
	Proteus spp (telephones)	[8, 12]
	Bacillus spp (telephones)	[8, 12]
Aerospace	*Staphilococcus* sp.	[16, 18, 22]
	Bacillus sp.	[16, 18]
	Corynebacterium sp.	[16]
	Enterococcus sp.	[16, 22]
	Micrococcus strains	[16, 18]
	Streptococcus sp.	[16]
	Aspergillus sp.	[16, 22]

Penicillium sp.	[16, 18]
Cladosporium sp.	[18]

A brief summary of the most common preventive or treating strategies for each application field is reported in Table 2.

Table 2: Preventive or treating strategies against bacterial contamination in various application fields

Application field	Strategies against bacterial contamination
Medical	Antiseptic operative procedures Antibiotic prophylaxis Revision surgery
Food handling	Cleaning , disinfection and sterilization [21, 27] Easy to clean design of equipment [27]
Everyday life	Cleaning and decontamination with chemicals (e.g. isopropyl alcohol and chlorexidine, disinfectant wipes) [7, 8] UV-light sanitizer/decolonizing treatments [7, 8] Antibacterial covers for telephones [7, 8]
Aerospace	Surface treatments (heat radiation, chemicals) [14] Cleaning surfaces Disinfectant wipes [14] Replacement of contaminated surfaces [14]

1.2 The antibacterial effect of silver

Silver is a metal able to expressed an antibacterial action towards a broad-spectrum of Gram-positive and Gram-negative bacteria and fungi [29-31]. Valuable pictures about the antibacterial effects as well as the impacts on human health and environment of silver-containing materials have been recently reported by Marambio-Jones and Hoek [32]. The action mechanism of silver against cells depends on its nature as ion, metallic silver or nanoparticle [33-35]. Even if the interaction mechanism is still not completely understood, silver generally reacts with thiol groups present into specific chemical sites as enzymes or proteins or DNA, altering or inhibiting the function of them (i.e. respiration, inactivation or replication processes) and causing the cell death. Metallic silver in a biological environment forms ions which play the main role in its antibacterial activity. Silver ions interfere directly with proteins or enzymes or DNA because they can attach and break cell membranes and enter into the cell nucleus. On the other hand, silver nanoparticles became attractive antibacterial agents during the last years due to their unique properties. The antimicrobial activity mechanism of silver nanoparticles is very similar to that of metallic silver, but enhanced thanks to their large surface area which provides a higher amount of released silver ions and a better contact with cells [29-31, 33, 35]. Moreover a direct action of silver nanoparticle on bacterial cells has been documented [29, 32]. For the same reason, silver nano-particles may result toxic and hazardous for the environment and human cells because of several peculiar nanoparticles factors as size, shape, chemistry and so higher reactivity and the environmental features as pH, ionic strength, presence of ligands [32, 35-37]. Few toxicity data are available for silver and less about the behavior of silver nanoparticles, despite a significant increase in their application in various fields, resulting in human exposition to silver by numerous routes, mainly ingestion, inhalation and skin contact. The main heath effect caused by chronic exposition to silver is argyria (the permanent grey/blue coloration of the skin), however silver accumulation in tissues and organs can be documented in some cases [35].

In the last few years, researchers developed different silver-based coating processes able to confer antimicrobial properties to almost all materials; the use of silver-based products may currently cover a variety of application fields from the treatment of burned wound [38], to medical devices [39-43], textile fabrics [44, 45] and water purification [46].

Silver-related antibacterial properties can be conferred to a surface using several different techniques. Silver can be added to glass and ceramic directly during melting process [47] or through ion-exchange [48-53], absorbed on zeolites or in silica microsphere [54, 55], deposited as coating on different substrates deposited by means chemical vapor deposition [56], sputtering [57, 58], or embedded into materials through sol-gel method or in the polymeric monomer or matrix [59-63]. Some of these methods are low-cost and available at an industry level, but they could not be applied on all kinds of materials as for instance, to polymers, because of the high temperatures necessary during the process or needed additional treatment for stabilizing the silver doped materials or coating.

Considering the widespread diffusion of silver-based antibacterial products, silver or silver nanoparticles accumulation in the environment and the consequential pollution and toxicity concerns must be considered, on the other hand few information can be found on these topics [35]. In this context the stability of silver containing products and their release kinetics (both for ions and nanoparticles should be considered) should be taken into account.

2. SPUTTERED ANTIBACTERIAL SILVER NANOCLUSTER/SILICA COMPOSITE COATINGS [n3]

In this section of the chapter, an innovative silver nanocluster/silica composite coating, developed and studied by authors to confer antibacterial properties to several materials is reviewed. First of all, a brief the sputtering technology used for the deposition of this coating is briefly described. Then, the main properties and characteristics of the silver nanocluster/silica composite coating, evaluated on a silica substrate used as model, are discussed. Finally, some case studies such as antibacterial biomedical devices, telephones, food containers and space structures are reported.

2.1 Sputtering technology

From the second part of the last century the thin film technology has rapidly grown due to the progress in vacuum systems and electronics.

The sputtering technology is the most diffused one in the electronic thin films industry and currently its use is increased also in biomedical, mechanical and other industrial sectors. Sputtering is one of the Physical Vapor Deposition (PVD) techniques, but it differs from others because it is a "not-thermal" process [64]. The sputtering process is realized in a vacuum chamber, consisting in an ion bombardment of the material to be deposited (target) that extract atoms or cluster of atoms. The atoms "fly" inside the depositions chamber and reach the substrate material, according to their free mean path. The deposited thin film starts its growing, regulated by the chemical and physical affinity between ad-atoms and substrate. During sputtering, the target is "vaporized", exploiting the momentum transfer among gas ions (usually Ar^+) and the target surface. The released energy is generated by atomic mechanical interaction and not by thermal heating, thus without breaking the intra-molecular bonding [65].For this reason, sputtering is used to deposit composite materials or in general molecular compounds like SiO_2. By avoiding melting, the target stoichiometry is almost unchanged from the target to the sputtered coating. Moreover, the control of many parameters such as plasma energy and carrier gas pressure during sputtering allows the optimization of conditions for each kind of film composition. Another advantage of sputtering is the film quality: as the sputtered atoms (or molecules) have generally greater energy if compared to those obtained by other "thermal" techniques, it results in an improvement in terms of film adhesion and cohesion. The fine tuning of parameters allows to better control uniformity and film thickness. The process stability during time and along the whole target surface guarantess the uniformity of the sputtered layer (on a nanometeric scale) even on high area samples (square meters). Finally, the sputtering process is a “green” one, with exhaust gases generally not toxic or hazardous and in a very low quantity. Only the initial equipment cost can be an issue.

An innovative antibacterial coating was developed to confer antibacterial behavior to most of the used materials (glasses, ceramics, metals, polymers) [66-68]. Starting from two different targets, one of pure SiO_2 which forms the matrix and one of Ag that provides the antibacterial effect, the thin film is realized by tailoring the SiO_2/Ag ratio in order to optimize its antibacterial behavior and mechanical / thermal properties.

Due to the low depositions rate of silica, high power density on this target is required and low energy is needed on the Ag target. Moreover to better control the SiO_2/Ag ratio a duty cycle during deposition is applied on the Ag cathode by switching on and off the plasma on the Ag cathode during the whole deposition time. By increasing the power on the silica target, the SiO_2 deposition rate, the substrate temperature and the thin film strength increase. On the contrary, by increasing the power on the Ag target, the substrate temperature is generally not affected because the Ag does not require high energy to be deposited.

In order to increase the antibacterial effect, the amount of Ag must be increased, but it must be also balanced to guarantee the film cohesion.

The deposition time affects the film thickness and consequently the total amount of Ag into the layer. Moreover, by using a non-cooled substrate holder and increasing the deposition time increases the substrate temperature: this can be dangerous for polymer substrates.

The antibacterial effectiveness is also related to the Ag ions release: it is thus evident that by increasing the Ag content in the film, it is possible to increase the lifetime of the antibacterial coatings.

2.2 Properties of the silver nanocluster/silica composite coating

The co-sputtering technique allows the simultaneous deposition of silica and silver, forming silver nanocluster/silica composite coating. In this paragraph, the main chemical, antibacterial, thermal and mechanical properties of the silver nanocluster/silica composite coating, studied and developed by authors [69-72], are reported.

The analyses are carried out on the silver nanocluster/silica composite coating deposited on a silica substrate. It was, in fact, demonstrated that silica is an inert material which does not interact with the coating chemical properties, allowing determination of the principal characteristics of the silver nanocluster/silica composite film [72].

The thickness of the coating is selected at 300 nm for these discussion. The coating thickness is an important parameter directly correlated to the total silver content, which is in turn related to the antibacterial effect. Hence, the choice of the thickness and the optimization of the silver amount are important aspects, especially for biomaterials which are close to body organs and tissues, with an increased risk of cytotoxicity development, as it will be discussed in the next chapter.
In addition, as the final application of an antibacterial layer could require high temperature, the effects of temperature between 300 and 600°C are evaluated subjecting the silver nanocluster/silica composite coating to several thermal treatments: results are reported here compared with the as deposited coating.

The silver nanocluster/silica composite coating deposited on silica substrate has a typical homogenous dark brown color (Figure 1a).

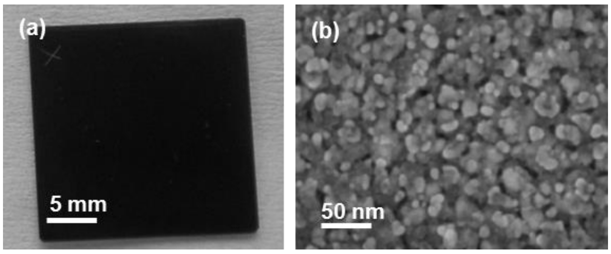

Figure 1: silver nanocluster/silica composite coating on silica substrates: (a) photograph, (b) FESEM micrograph

Observing the coating morphology reported in the field emission scanning electron microscopy (FESEM) micrograph in Figure 1b, the silica matrix has the typical porous structure of a sputtered layer whereas silver nanoclusters (few nm) as bright dots are well embedded in it. The matrix is able to firmly hold the silver nanoparticles and to release only silver ions, principal responsible of the antibacterial effect. The composite nature of this coating reduces the amount of silver necessary to obtain a coating, if compared to the amount of silver needed to obtain a metallic silver layer, thus reducing toxicity issues and overall cost.

The antibacterial activity against *Staphylococcus aureus* of the silver nanocluster/silica composite coating is demonstrated by means of inhibition halo test [73] as reported in Figure 2.

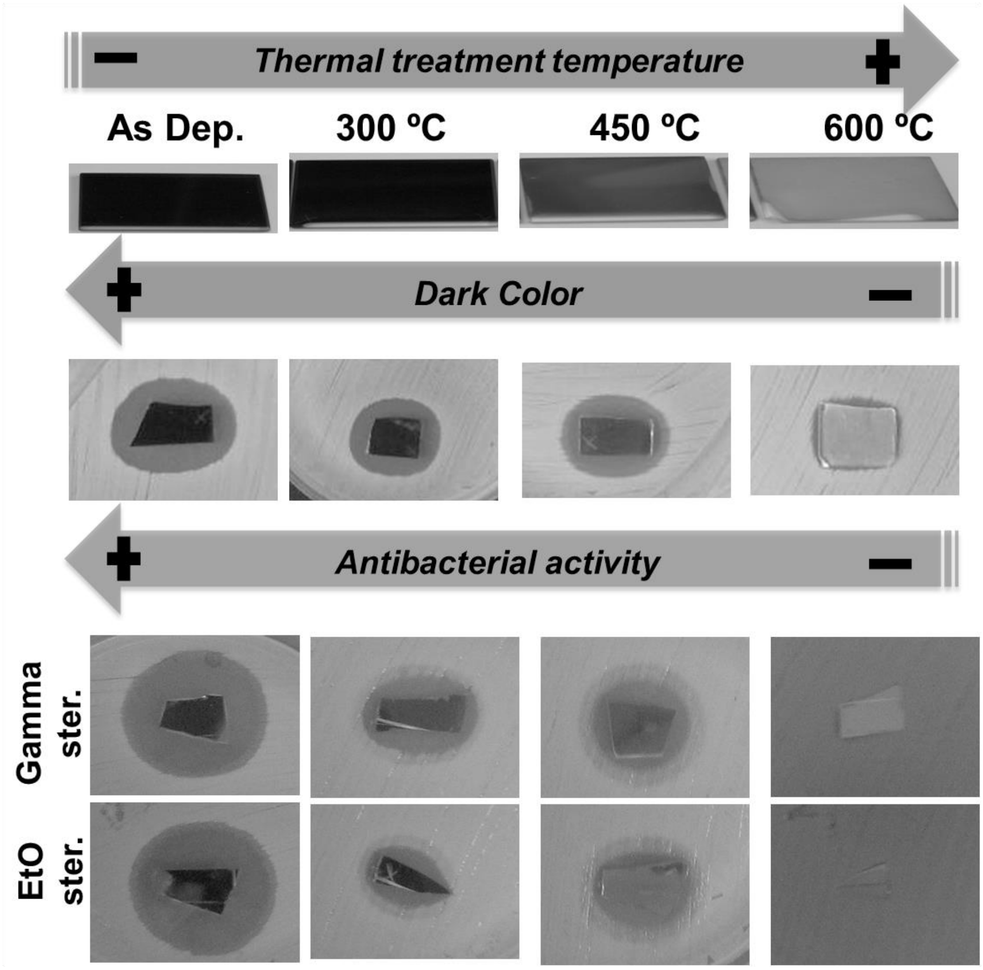

Figure 2: Effect of the thermal treatment and sterilization on visual appearance and antibacterial behavior of the silver nanocluster/silica composite coating on silica substrates

Silver nanoclusters in the as deposited coating (As dep), generate silver ions which diffuse into the agar and produce a well visible halo of about 5 mm. The thermal treatments up to 450°C do not influence the antibacterial behavior and the halo formed around heated samples remains quite the same, well visible and reproducible (test performed in triplicate). On the contrary, a reduction of antibacterial effect is visible for coated samples heated at 600°C, where the inhibition halo is less evident. However the small inhibition halo is sufficient to give an antibacterial effect. This feature can be explained by considering the mechanism of antibacterial activity directly related to the particles dimensions and to the surface area vs volume ratio [74]. If the particle size is small, the antibacterial effect is more efficient because the surface area vs volume ratio is larger. A nano-particle size increase, together with the total number of nano-particle decrease occurs with the thermal treatment at 600°C, thus reducing the whole antibacterial properties. For the same reasons, silver nanoclusters tend to coalesce involving a color change with a gradual shift from dark as deposited coatings to light, almost transparent yellow/orange ones heated at 600°C. As explained in Refs. [69, 75, 76], the scattering and Localized Surface Plasmon Resonance (LSPR) effects are due to the silver nanoclusters embedded in the silica matrix as a function of their size. The dark colour is the effect of small nanoclusters, because of the prevalence of the scattering effect. On the other hand, a light colour (yellow/orange) is typical of the larger nanoclusters where the silver LSPR at 400 nm is predominant.

The sterilization procedures, performed on both as deposited and heat treated samples with EtO (Ethylene Oxide) or gamma rays, both widely used in the biomedical field, do not affect antibacterial activity and visual appearance.

The silver nanoclusters size increase discussed above is also verified through XRD analysis (Figure 3) where the intensity of metallic silver peaks significantly increases.

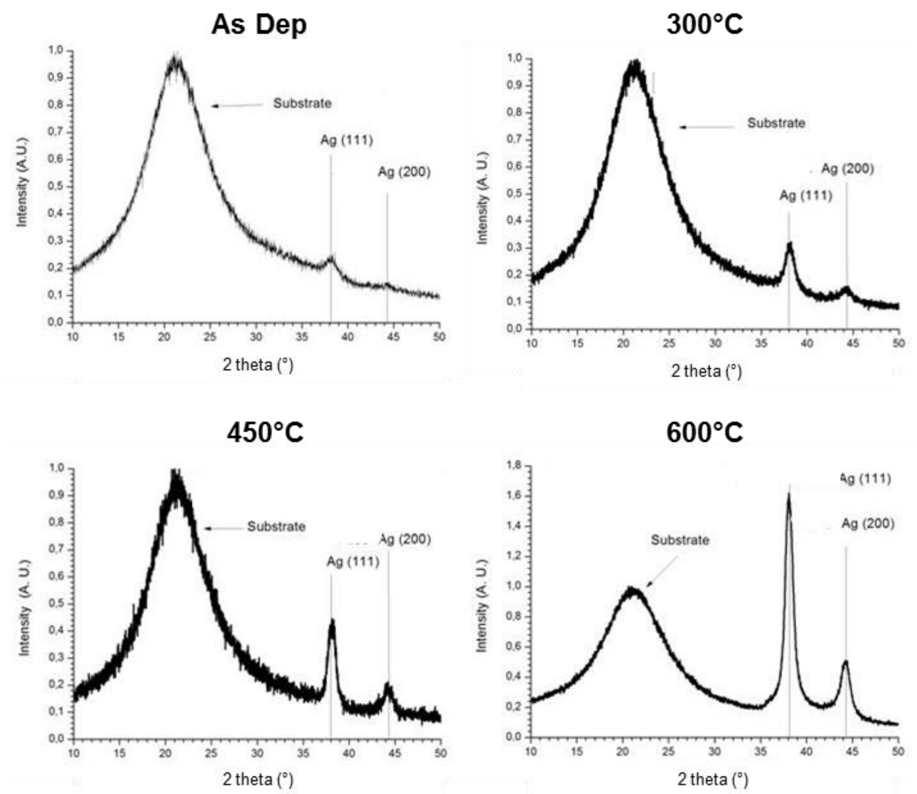

Figure 3: XRD of as deposited and thermal treated silver nanocluster/silica composite coatings on silica substrates[n4]

The effect of temperature on the antibacterial behavior of silver nanocluster/silica composite coatings on silica are confirmed also through metal ions release (leaching) test. The leaching test performed by dipping both as deposited and heat treated coatings into distilled water at 37°C up to a month then analyzed by means of a graphite furnace atomic absorption spectroscopy, shows a gradual decrease of the silver ion release with the coating heat treatment temperature increase (Figure 4). This behavior confirms what previously discussed about the role of surface vs volume ratio on silver nanoclusters properties.

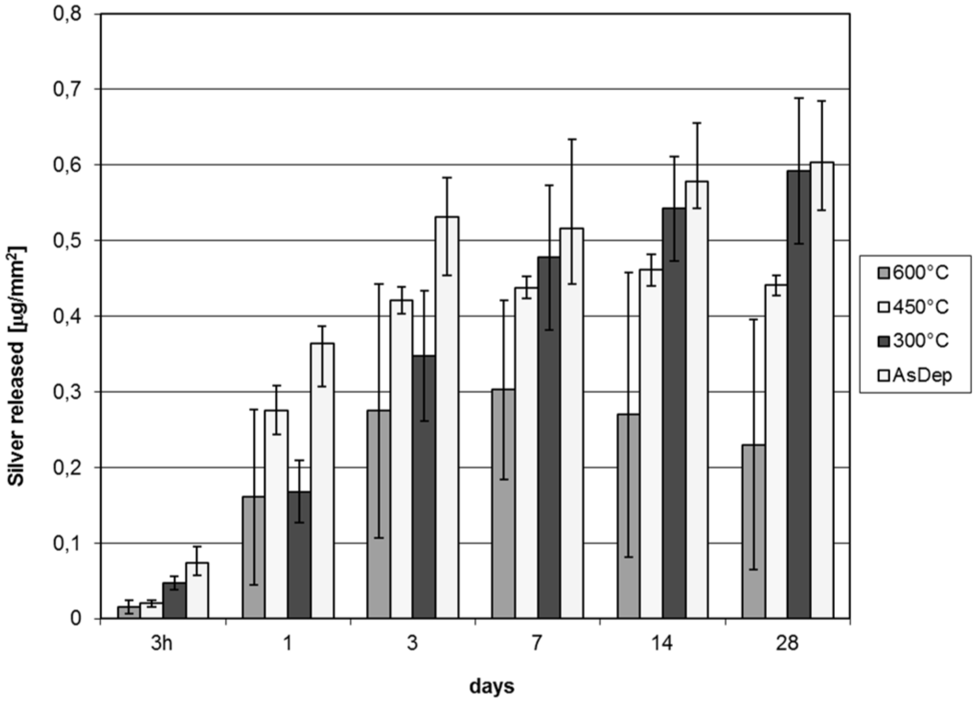

Figure 4 Silver ions release in water at 37°C from as deposited and thermal treated silver nanocluster/silica composite coatings on silica substrates

Besides the antibacterial behavior and the chemical stability of the coating, mechanical properties have to be considered. In general, silver nanocluster/silica composite coatings show good adhesion to silica substrates both as deposited and after treatment up to 450°C [69, 70]. After the tape test performed according to the ASTM D3359-97 standard [77], the coatings do not present damage or sign of detachment form silica substrates.

The nano-hardness are measured on as deposited and heat treated coatings and reported in Table 3, the results compared with those obtained from the silica substrate. The nano-indentation tests were performed by keeping constant either the maximum indentation depth (30nm or 50nm) or the maximum applied load (1mN and 5 mN) [71].

Table 3: Nano-hardness results

Samples	**Hardness (GPa)**			
	maximum indentation depth		**maximum applied load**	
	30nm	**50nm**	**70-80nm**	**190-200nm**
	0.3mN	**0.6mN**	**1mN**	**5mN**
Silica substrate	51 ± 8	40 ± 3	24 ± 1	**12 ± 0.4**
As dep coating	22 ± 4	15 ± 2	12 ± 1	9 ± 0.2
TT300°C coating	26 ± 3	16 ± 1	12 ± 1	9 ± 0.3
TT450°C coating	34 ± 6	20 ± 1	17 ± 1	**11 ± 2**

As expected, the as deposited coating presents a lower nano-hardness than pure silica substrate. Increasing the heat treatment temperature the hardness increases: this is more evident at low loads, whose result corresponds to the first nanometer of sputtered coating, comparable to that of the silica substrate.

1. APPLICATIONS AND CASE STUDIES [n5]

1.1 Biomedical devices: Polymers

The bacterial contamination and the infection development of biomedical devices are currently a well-known and problematic issues. The use of antibiotics and the introduction of strict hygienic protocols have remarkably minimized the risk of infection; however bacterial contamination can cause the failure of the implant, increase the hospitalization time , need of a new surgery, thus leading to patient's pain and even death. Moreover bacteria, responsible of infections are continuously developing increasing resistance to antibiotics, so new biomaterials with antibacterial capability are necessary.

Silver is widely used and studied as antimicrobial agent also in biomedical field, for this reason silver nanocluster/silica composite coating were sputtered on different polymeric materials such as poly(methyl methacrylate) (PMMA). This recent application, disclosed in a patent by the authors [20], involves the deposition of silver nanocluster/silica composite layers on selected critical surfaces of orbital implants and ocular prostheses, in order to reduce the risk of postoperative infections in enucleated patients. In order to preserve the polymer thermo-mechanical resistance, the parameters of the sputtering process were investigated and adapted to the polymers substrates. Moreover, silver content was optimized to avoid possible toxic effect according to the final application.

Figure 5 reports the visual appearance of sputtered PMMA (a), the morphology of the coating observed by FESEM (b) and the antibacterial inhibition halo formed by the coated PMMA (c). Both the dark color and the morphology of the coating deposited on PMMA is the same one observed on the silica substrate (Figure 1) with the typical porous morphology of sputtered silica. The antibacterial behavior is demonstrated by means of the formation of an inhibition halo of about 5 mm around the sample (Figure 5c).

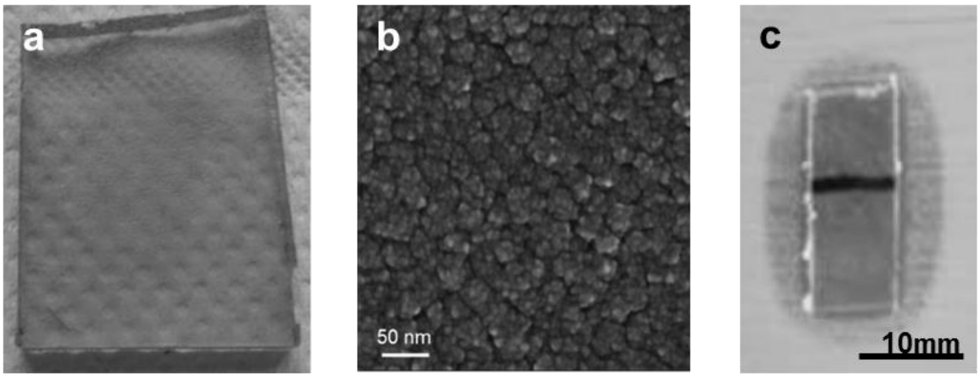

Figure 5: Silver nanocluster/silica composite coating on PMMA sample : visual appearance (a), FESEM image (b) and antibacterial inhibition halo (c)

Similar results were obtained for other kinds of polymers typically used in biomedical fields as polyurethane and polypropylene. Also in this case, the antibacterial behavior is successfully conferred to the material through the deposition of silver nanocluster/silica composite coating by means of the co-sputtering technique.

3.2 Food handling and domestic appliances: cheese molds and mobile phones

Microbial biofilm formation is a topic of considerable interest in facilities or services daily handled as in the food industry or belonging to the every-day human life as mobile telephones.

Many microorganisms are able to easily form biofilms on *food containers*, thanks to the suitable environment conditions even if cleaning and disinfection procedures of surfaces are accurately performed to prevent the microbial colonization. For example, in the case of cheese industry, bacteria are present in the the raw milk, milking machines, farm environment, cheese processing plants and also from operators [9-11]. Hence, stainless steel for cheese molds was used as substrate for silver nanocluster/silica composite coating (Figure 6a). The inhibition halo of about 5 mm against *S. aureus* is formed around the sputtered steel as in Figure 6b.

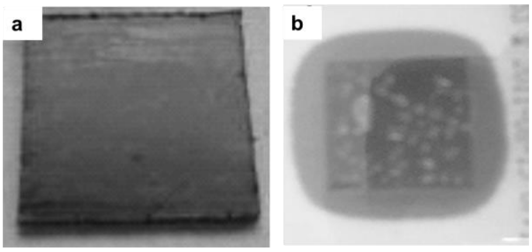

Figure 6: Steel for cheese container: a) coated steel and b) inhibition halo formed against S. aureus

The effect of the thermal treatments is evaluated also with this substrate. The enlargement of nanoclusters size and the consequent chromatic change occurs as observed for the silica substrates. In addition, the adhesion of coating to steel is improved after heating.

Since *mobile telephones* are manipulated by billions of people every day and they come in contact with human body parts, as ear, hand, mouth and hair, they are considered as important carriers of bacteria. For this reason, the antibacterial silver nanocluster/silica composite coating was sputtered on several polymers (polycarbonate, blend polycarbonate- Acrylonitrile Butadiene Styrene ABS, polyester monofilament fibre) typically used in mobile telephone components such as screens, covers and microphone felts. Table 4 reports the materials used for the different mobile telephone parts.

Table 4 : Polymers used in mobile telephones

Mobile telephone part	Material
Protective lenses for screen	polycarbonate (Sabic Innovative Plastics, Lexan™)
Cover	polycarbonate yellow cover of Samsung™ S3650
Cover	blend polycarbonate-ABS black cover of Nokia™ 1616
Protective tissue felts for electro-acoustic transducers	polyester monofilament fibre Saatifil Acoustex™

Different sputtering conditions were applied to adapt silver concentration and coating thickness in order to satisfy strict aesthetical, transmittance requirements and antibacterial properties of each mobile telephone part.

Figure 7 shows the visual appearance of a coated screen (a) that maintains the transparency requirements, the coated cover (b) and the coated felt (c).

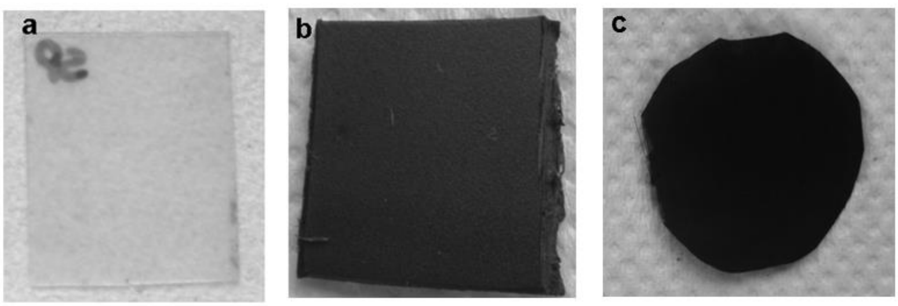

Figure 7: different sputtered parts of a mobile phone : a) screen, b) cover, c) felt

The antibacterial activity is tested through the count of adhered CFU [78] comparing the results of sputtered and not sputtered materials (Figure 8). The count of CFU in Figure 8a demonstrates the coating ability to reduce the bacterial contamination on the coated screen. The number of bacteria is reduced of two orders of magnitude with respect to the uncoated screen.

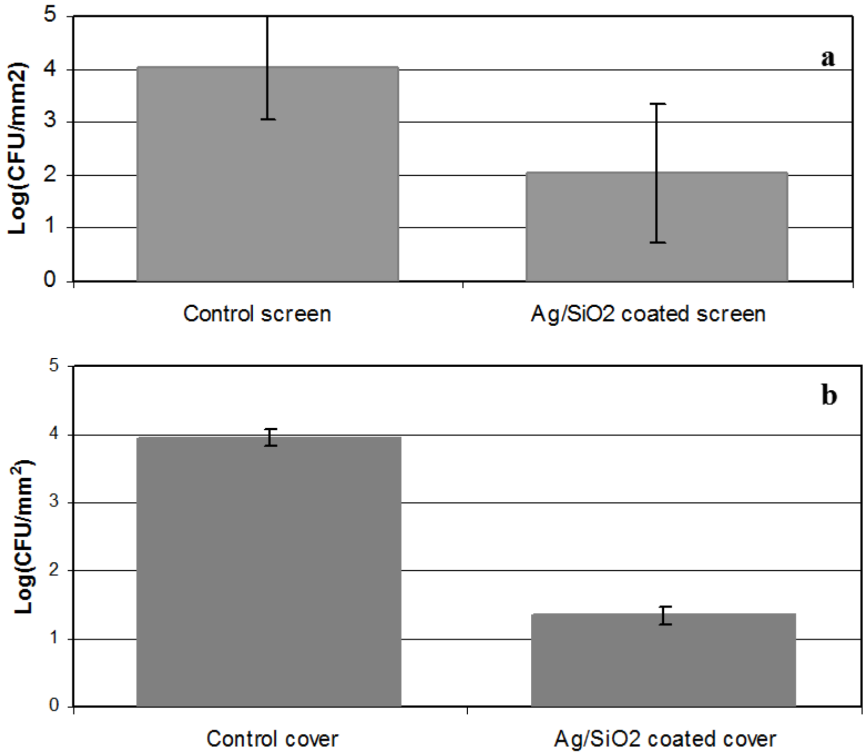

Figure 8 : CFU count for sputtered materials compared with the uncoated ones: a) screen and b) cover

Figure 8b demonstrates that also the coated covers decreased the bacteria number by 2–3 orders of magnitude if compared to the as received control cover.

Acoustic felts consist in black fabrics hidden in small holes in the telephone, so they do not require specific aesthetical properties. However, it is fundamental that the acoustic performance of the material remained unaltered after the coating deposition. In addition, the risk of bacterial contamination is very high because they are continuously exposed to mouth and breath and they are difficult to clean. Felts coated with the silver nanocluster/silica composite coating are able to provide a reproducible and clear inhibition zone (Figure 9) of about 3-4 mm and no alterations of acoustic performance were detected.

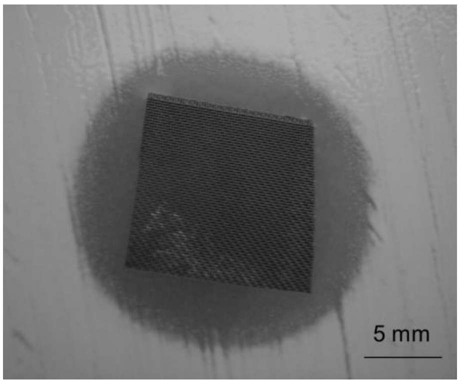

Figure 9: Antibacterial inhibition halo around sputtered felt

The different telephone parts coated with the antibacterial layer were assembled and the obtained antibacterial mobile telephone was perfectly working.

3.3 Aerospace: Combitherm ®, Kevlar ®, Aluminium alloys

During the most recent space voyages, an environmental biocontamination has been discovered on board of the International Space Station and Mir orbital station [14, 16, 18]. The space structure habitat is characterized by conditions as temperature, humidity and presence of humans in a manned place with limited comforts, optimal for an easy microorganisms colonization and incubation of surfaces, instruments, potable water containers and air conditioners [14-16]. The development of a microbial film induces not only an increment in medical risks and pathogenic effects but also a reduction of the structures integrity because of materials deterioration [22, 23, 79]. Polymers and metals tend to corrode more quickly for the microorganisms colonization than in normal conditions [22]. The bacterial and fungal contamination, studied and controlled during the most recent space activity, results delimited and below the acceptable limits, thanks to controlled prevention, monitoring and disinfection methods [14]. Anyway, the current procedures could be not enough to guarantee crewmembers' health, safety and wellbeing, especially in prolonged space exploration, together with ensuring the structures safety.

For this purpose, silver nanocluster/silica composite coating is optimized for substrates suitable for the realization of a prototype of space inflatable modules [80] without altering gas retention and mechanical properties [81]. In particular, the antibacterial coating is deposited on the polymers (Combitherm®, a multilayer polymeric film with polyethylene as the most external layer, and aramidic fabric) used for building the most internal wall, strictly close to the crewmembers, and on aluminum alloy selected for the structural block. Figure 10 reports the photographs of the two polymers before and after coating deposition and the inhibition halo formed against *Staphylococcus aureus*.

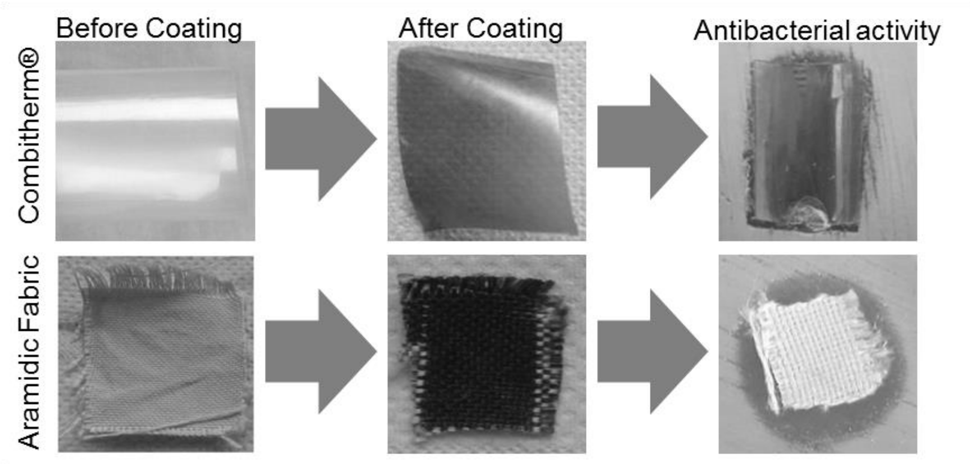

Figure 10: Polymers used in the realization of inflatable space structure, Combitherm® and aramidic fabric, before and after deposition and the antibacterial inhibition halo

After the coating deposition, the surface of both polymers becomes of dark brown color due to the LSPR effect of metallic silver, as previously explained. The Combitherm® film is susceptible to the exposition for long time at temperature about 80°C as that reached into the sputtering chamber. Hence, a "softer" deposition process (deposition time 15 min, coating thickness 60 nm) than that applied to the aramidic fabric (80 min) is needed to avoid substrate deterioration. Both the coated samples induce the formation of an inhibition halo which demonstrates the antibacterial behavior of the silver nanocluster/silica composite coating also on these substrates (in Figure10- the aramidic fabric is not transparent and the coated side of the aramidic fabric in contact with the bacteria agar is not visible). The bacteria free zone around aramidic fabric results more evident thanks to a thicker coating and consequently to a higher amount of available silver.

As discussed in [81], the efficiency of this antibacterial coating on Combitherm® substrates is more evident towards other bacterial and fungal species, i.e. *E. coli*, *B. cereus* and *Candida*, which are more sensitive to silver ions because of a different structure of their cell's wall .

Obviously, the sputtering deposition should not cause substrate deterioration: in the case of Combitherm®, the air permeability and the mechanical properties as tensile [82], tear [83] and perforation [84] resistance were tested before and after coating deposition [81]. In particular the permeability remained unchanged with a value of about 2 ml*mm/m^2*day*atm, a result considered suitable for the final application of this polymer as air bladder container. The antibacterial coating improves the tensile and perforation resistance whereas it does not affect the tear strength. Finally, as reported in Ref. [81], the silver nanocluster/silica composite coating results well adherent to the Combitherm® substrate without sign of detachment after tape test [77] and it is able to resist up to 3000 laps during the abrasion test [85]against an aramidic fabric.

Aluminum alloys for aerospace application are also used as substrates for the deposition of the silver nanocluster/silica composite coating. The sputtered alloys are included into two international experiments where they are exposed in an environment with conditions suitable for the microbial film formation. In the first test, called MARS 500, concerning the simulation of a travel to Mars, the samples remained into a close environment for 520 days with the presence of crewmembers and with typical conditions of a space mission (Figure 11) [86]. In the second test named VIABLE, the sputtered samples has been sent on the International Space Station for evaluating the bacterial contamination for 4 years, together with other antibacterial treated materials, prepared by other scientific groups [87]. In both cases, the results of the bacterial biofilm formation and proliferation are currently under evaluation.

Figure 11: Sputtered aluminum alloys for MARS 500 experiment

CONCLUSION

An innovative antibacterial coating composed of silver nanoclusters embedded into a silica matrix was sputtered on several substrates. The coating appears as a brown colored layer with a nanostructured morphology. All sputtered materials formed a well visible and reproducible inhibition halo or a reduction in the count of colony forming units towards S. aureus and other bacterial and fungal strains demonstrating their antibacterial properties. The silver nanocluster/silica composite coating results well adherent to all these substrates, also with the most flexible ones, such as polymers. Appropriate thermal treatments could be performed in order to improve the cohesion and the adhesion of the coating. The coating bleaching due to enlargement of the nanoclusters size occurs with the temperature increase.

Tailoring the parameters of the sputtering process allows the optimization of the coating, according to the substrate properties or the application requirements. This degree of freedom of the coating process permits the use of this coating in a wide range of application fields, from metals for food handling to polymers used in biomedical applications, mobile telephones or for the realization of space structures.

A thermo-sensitive polymer can be thus coated by a suitable sputtering process able to guarantee its antimicrobial behavior without being damaged by the process; the silver amount in the coatings can be modulated and controlled thus avoiding possible cytotoxic effects which may occur for devices in direct contact with human body fluids.

Compared to the other antibacterial coatings made of pure metallic silver, this silver nanocluster/silica composite reduces the amount of silver necessary to obtain a coating, thus reducing toxicity issues and overall cost. It is also stable in air up to 600 °C at least and resistant to the most common sterilization procedures.

In addition, this composite coating can be tailored to an efficient compromise between suitable amount of silver to provide antibacterial effect and silver release kinetic safe from a toxicity point of view. Recent studies suggest that silver nanoclusters release in form of ions and not nanoparticles is a very important aspect considering the demonstrated toxicity of metal nanoparticles *in vitro* and *in vivo* experiments. Current investigations are being performed in order to study the mechanism of silver nanocluster release in this coating.

Moreover, durability of this coating in a prolonged contact with fluids is being investigated and other materials with higher stability than silica in a wet environment might be considered.

Finally, sputtering is potentially applicable on a large scale to substrates with irregular or curved geometries.

ACKNOWLEDGMENTS

This activity was funded by Regione Piemonte, Italy (NABLA, Nano-structured Antibacterial Layers) and by REA (EU Project-NASLA-FP7-SME-2010-1—Project 415 262209). The authors kindly acknowledge Dr Giacomo Fucale (Traumatology Orthopaedics and Occupational Medicine Department, University of Turin, Italy) for antibacterial tests facilities, Dr. Andrea Coggiola, Dr. Daniela Dolcino, and all partners of the projects (Thales Alenia Space—IT, Dipromed, Alce Calidad, Easreth, Aero Sekur, Reply, KTH Royal Institute of Technology, Bactiguard).

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